PARTHENOGENETIC DEVELOPMENT OF THE EGGS AND EGG FRACTIONS OF ARBACIA PUNCTULATA CAUSED BY MONOCHROMATIC ULTRA-VIOLET RADIATION

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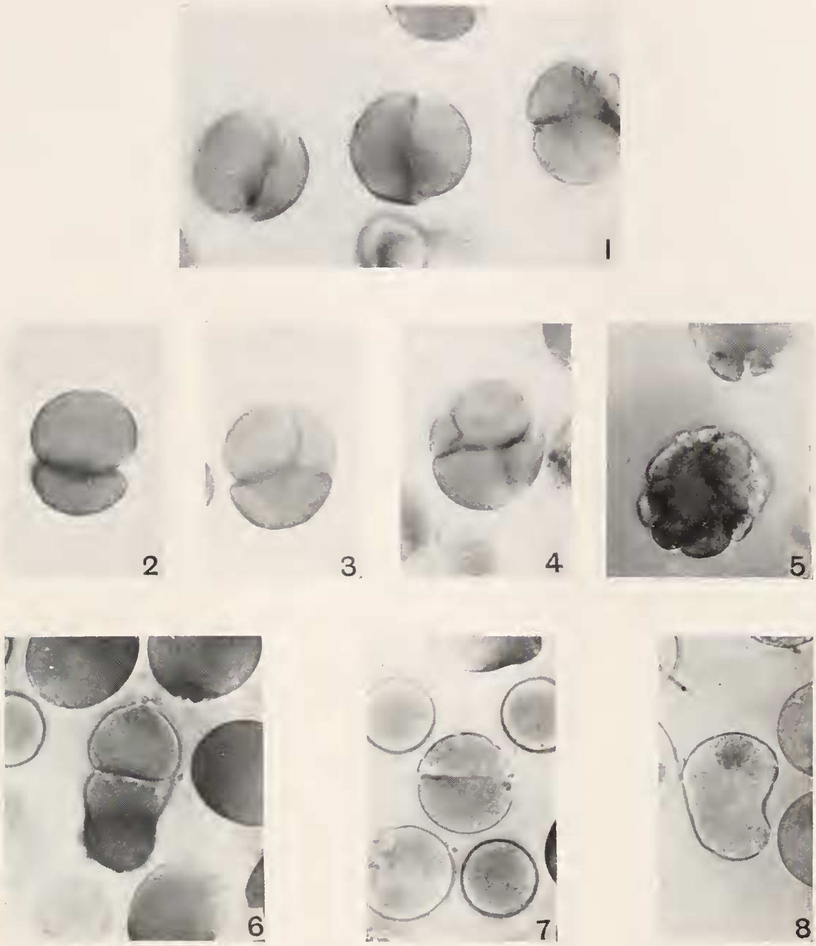
Normal unfertilized (uncentrifuged) eggs of *Arbacia punctulata* can be activated by ultra-violet radiation, either by the full arc for 1/10 to 10 seconds or by monochromatic radiation of 2260–2480A for two to eight minutes. The method has been fully described in the preceding paper in this journal (Hollaender). The eggs throw off a normal fertilization membrane and pass through several cleavages (Photographs 1–5). The cleavages are much delayed in comparison with normal fertilized eggs[1] just as they are after treatment with other parthenogenetic agents, such as hypertonic sea water. In some experiments, with the optimum treatment, all the eggs are activated and after five hours, 80 per cent have cleaved (Photograph 1); frequently the first cleavage gives two unequal cells (Photograph 2). In many eggs a second cleavage follows in an hour; it may be asynchronous in the two blastomeres resulting in a 3-cell stage (Photograph 3), or synchronous giving a typical 4-cell stage (Photograph 4). Further cleavages result in 8 to 16-cell stages (Photograph 5), but no further development was observed.

Whole eggs which have become stratified and elongated by a strong centrifugal force (10,000 $\times$ g for 3 minutes) are activated by the same radiation as the uncentrifuged eggs; they form fertilization membranes following the irregular contour of the egg, just as similar fertilized eggs do, or as do those artificially activated by hypertonic sea water. About 50 per cent of the eggs cleave, in about the same time as the uncentrifuged—that is, after four to five hours. The first cleavage plane usually comes across the short axis of the elongate egg (Photograph 6) just as it does in fertilized or other parthenogenetic eggs of the same shape (E. B. Harvey, 1932, 1936). When the centrifuged egg is spherical or nearly so at the time of radiation, the cleavage plane tends to come in through the equator, just as in normal fertilized eggs. The centrifuged eggs which have been radiated pass

[1] First cleavage of the normal fertilized egg takes place in 50 minutes at 23°.

PLATE I



Photographs of living *nucleate* eggs, taken with a Leica camera and water immersion lens.

1. Unfertilized uncentrifuged eggs, six hours after radiation of 2380A for five minutes. All eggs in field cleaved.

2. Same, 2-cell stage unequal. Cf. Photographs 20 (red half) and 26 (yolk quarter).

3. Same, seven hours after radiation. Three-cell stage. Cf. Photographs 21 (red half) and 27 (yolk quarter).

4. Same, 4-cell stage. (Cf. Photograph 28.)

5. Same, eight hours after radiation. Sixteen-cell stage. Cf. Photograph 23 (red half).

6. Centrifuged whole egg, six hours after radiation with full arc five seconds. Two-cell stage. Cf. Photographs 30, 31 and 36 of E. B. Harvey, 1936.

7. White half-egg, same treatment. Two-cell stage.

8. Another white half-egg, five hours after radiation. Nuclear membrane has just broken and egg is somewhat amoeboid.

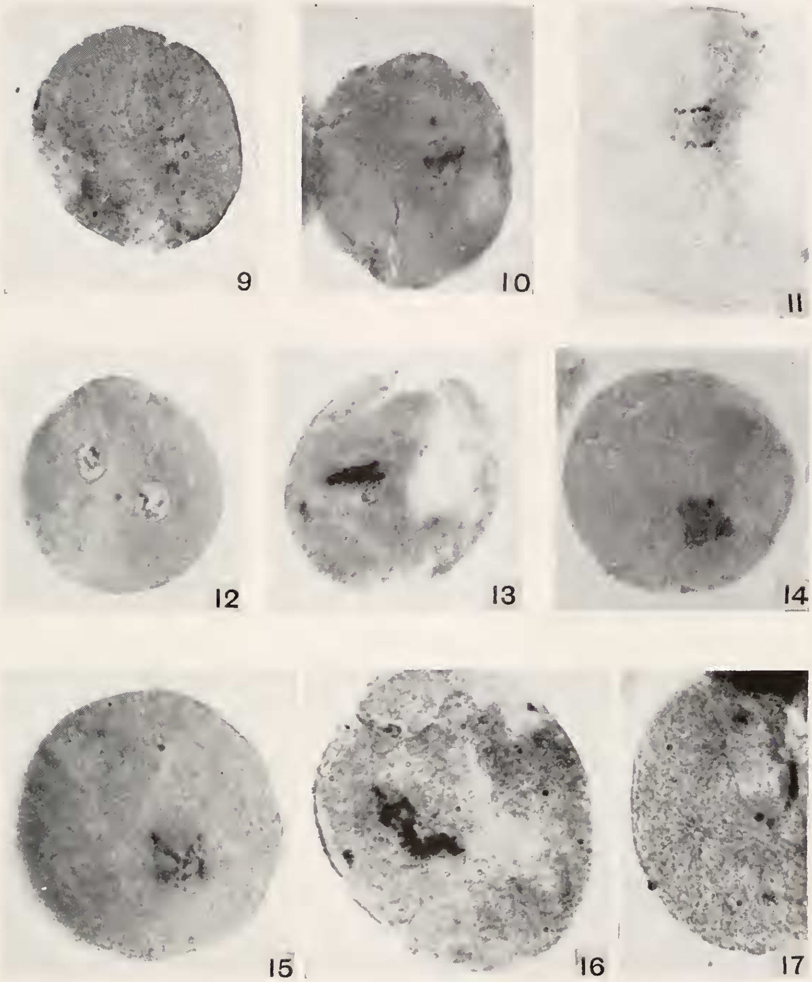
through several cleavages, but stop at about the same stage as the uncentrifuged.

The nuclear changes leading up to first cleavage can be observed in the living egg, especially in the centrifuged egg. After radiation, the usual nuclear changes take place, the enlargement of the nucleus, breaking of the nuclear membrane and the formation of the amphiaster; it is a perfectly normal cleavage in many observed cases. Prepared sections of later stages (8 hours after activation) of radiated uncentrifuged eggs showed some normal prophase, metaphase and anaphase figures (Photographs 9, 10 and 11). Typically there are no asters at the poles of these spindles (Photographs 10, 11) but occasionally they are well-formed (Photograph 9); and large asters may occur independently of chromosomes, in the cytoplasm (Photograph 17). Spindles without asters are characteristic of somewhat later cleavages of the normal fertilized *Arbacia* egg when the cells are very small, but in the early stages corresponding to those pictured here, the asters of the normal dividing egg are very conspicuous. Photograph 12 shows the quite normal-looking nuclei resulting from the first division of a radiated egg. Together with the fairly normal mitotic figures, and in much greater abundance, are abnormal figures. The most characteristic feature of the radiated eggs is the occurrence of multipolar spindles, each spindle with its chromosome plate. The axes of the spindles may be parallel (Photographs 13, 14) or they may lie at any angle with regard to each other, forming a rather confused figure (Photographs 15, 16). The chromosome number appears to be sometimes haploid and sometimes diploid in the more simple type of spindle, but is very large in the compound spindles, and judging from the formation of these spindles should be, at least in some cases, tetraploid (Photograph 14).

White half-eggs, obtained by breaking the whole eggs into two parts by centrifugal force,[2] are also activated by ultra-violet radiation. These are the lighter halves and contain the female nucleus. When activated, they form fertilization membranes, and usually after five hours cleave equally since they are spherical (Photograph 7), just as do white halves which are fertilized. Subsequent cleavages have given 8-cell stages, but no further development. These white half-eggs are much more sensitive to the same amount of ultra-violet radiation than are the whole eggs, normal or centrifuged. With the optimum treatment, only 5 per cent of the eggs had cleaved after five hours; those radiated slightly longer were all cytolyzed after five hours, and those radiated slightly less were not activated. The

[2] For a description of the method, see E. B. Harvey, 1932 and 1936.

PLATE II



Photographs of prepared and stained sections of whole uncentrifuged eggs, taken with Leica camera and oil immersion lens. The eggs were fixed seven hours after radiation with full arc for five seconds.

9. Normal prophase with asters.
10. Normal metaphase, but no asters.
11. Normal anaphase, but no asters.
12. Two-cell stage, with normal nuclei.
13. Beginning anaphase, several spindles combined, in same plane; side view.
14. Metaphase plates of four spindles combined in same plane.
15. Metaphase, several spindles combined in various planes.
16. Same, polar view.
17. Large cytasters without spindles or chromosomes.

"dose" is rather critical. There is a tendency for the white half-egg to become aspherical and amoeboid (Photograph 8).

Red half-eggs, the heavier halves obtained by breaking the whole egg into two parts by centrifugal force, do not contain a nucleus, nor any part of it; the nucleus goes invariably into the lighter white halves. The red half-eggs are, nevertheless, activated by ultra-violet radiation. They are activated by the same radiation as the whole eggs and also by a band of longer wave-length, 2650–3300A for four to twelve minutes, a treatment which does not affect the whole eggs and white halves, but does affect the yolk and pigment quarters. They form a fertilization membrane and a thick ectoplasmic layer (Photograph 18). After an hour and a half, a clear area is observed in the less dense portion, a sort of pool, just as in the red halves activated by hypertonic solutions (E. B. Harvey, 1936, p. 111). In a few cases, large monasters in this area are observed four hours after radiation. These have also been found in prepared sections of the red half-eggs (Photograph 31). A notch frequently appears at the equator of the more spherical red halves indicating the beginning of a cleavage plane (Photograph 19). Two-cell stages of these partheno-

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PLATE III

Photographs of living *non-nucleate* eggs taken with Leica camera and water immersion lens, except the last photograph, which is from a prepared slide taken with an oil immersion lens.

18. Red half-egg, six hours after radiation of 2380A for five minutes. Note fertilization membrane and ectoplasmic layer.

19. Red half-egg, seven hours after radiation, full arc 10 seconds. Indentation at equator.

20. Red half-egg, eight hours after radiation, full arc five seconds. Two-cell stage. (Cf. Photographs 1 and 2 of similar *nucleate* egg.)

21. Red half-egg, seven hours after radiation of 2380A for six minutes. Three-cell. (Cf. Photograph 3 of similar nucleate egg.)

22. Same lot. Four-cell.

23. Red half-egg nine hours after radiation of 2380A for six minutes. Eight to twelve-cell. (Cf. Photograph 5 of similar nucleate egg.)

24. Red half-egg seven hours after radiation of full arc five seconds. Amoeboid.

25. Same egg one-half hour later.

26. Yolk quarter seven hours after radiation of 3300A for five minutes. Two-cell. (Cf. Photographs 1 and 2 of nucleate egg.)

27. Same egg one hour later. (Cf. Photograph 3.)

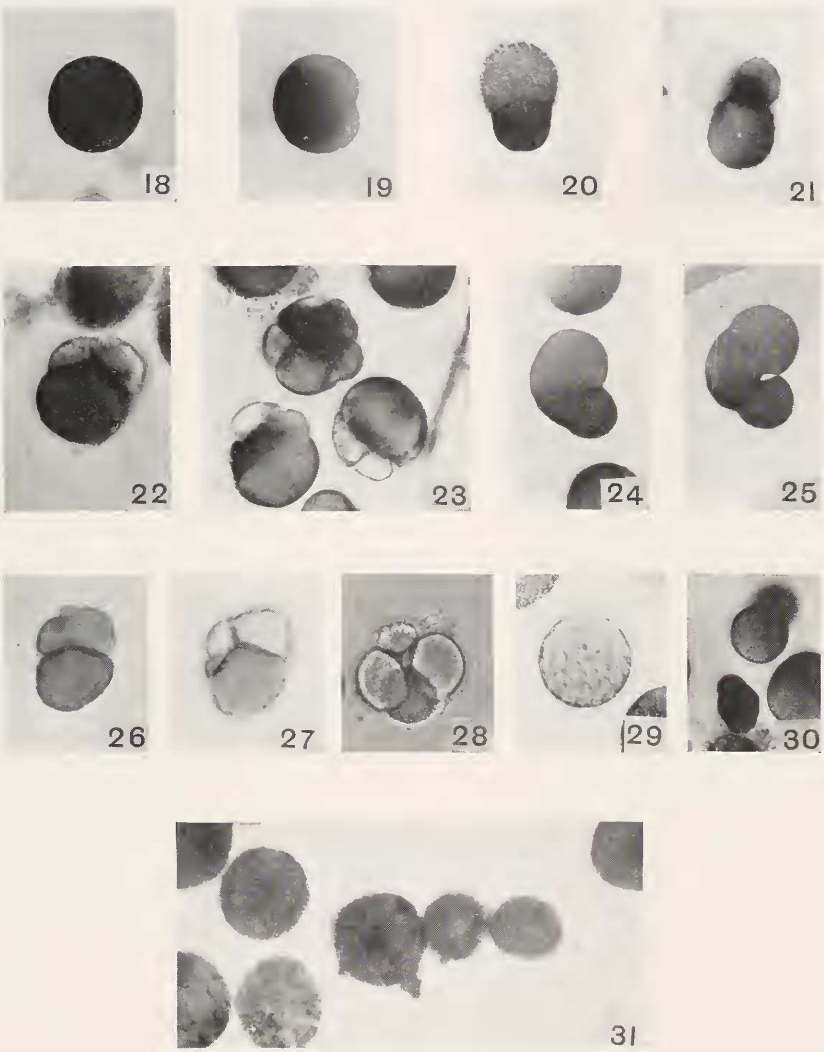
28. Yolk quarter eleven hours after radiation of 2380A for six minutes. Four-cell. (Cf. Photograph 4.)

29. Yolk quarter seven hours after radiation of 3300A for nine minutes. Multi-astral.

30. Pigment quarter (lower cell) seven hours after radiation of full arc five seconds. Amoeboid.

31. Prepared and stained section of red half-eggs seven hours after radiation with full arc for five seconds. Note large aster in center cell and absence of chromosomes and spindles. (Cf. similar nucleate cells of Plate II.)

PLATE III



genetic merogones occur (Photograph 20) and a further cleavage gives a 3- and 4-cell stage (Photographs 21, 22). A 12-cell stage is the latest that has been observed (Photograph 23). Later on, however, some of these eggs are found with many asters, a possible precursor to a blastula and similar to what has been found in red halves activated in other ways. The similarity of the development of the non-nucleate egg with that of the nucleate may be seen by comparing similar stages, such as Photographs 21 with 3, 23 with 5 and 28 with 4. Frequently the red half-egg, after radiation, becomes aspherical and somewhat amoeboid with a tendency to form and split off a lobe from the pigmented end (Photograph 24). This is sometimes completely separated by what appears to be a true cleavage plane (Photograph 25).

The yolk quarter and pigment quarter which are obtained by further centrifuging of the red half, and which are, of course, non-nucleate, are both activated by radiation of the same wave-lengths as the red halves. The yolk quarters cleave several times (Photographs 26–28) to give as many as eight or twelve cells. Multi-astral yolk quarters also occur (Photograph 29).

Pigment quarters, although only one-fourteenth the volume of the whole egg, show evidence of activation in that they form an ectoplasmic layer and become somewhat amoeboid (Photograph 30).

A few clear quarters (nucleate) and granular quarters (non-nucleate) obtained by further centrifuging of the white half-eggs also show signs of activation by radiation. The clear quarters become amoeboid and the nucleus breaks down, and the granular quarter forms a fertilization membrane and ectoplasmic layer. Owing to the difficulty in obtaining these fractions, only a few were radiated, and a thorough investigation was not made.

The foregoing results show definitely that ultra-violet radiation acts upon the whole eggs and their fractions obtained by centrifugal force exactly like other parthenogenetic agents such as hypertonic sea water. The most interesting and important result, however, is that the radiation activates the *non-nucleate* fractions of eggs. The action of the radiation must, therefore, be primarily upon the cytoplasm. The nuclear changes leading up to first cleavage, in the whole eggs, both normal and centrifuged, and in the white half-eggs, seem perfectly normal, but the later spindles and chromosome groups are often quite irregular and abnormal. The conclusion is drawn, then, that the ultra-violet radiation affects primarily the cytoplasm, but there is also an effect upon the nucleus. Whether this is a direct action of the radiation on the nucleus or a secondary effect due to changes first produced in the cytoplasm by the radiation, it is difficult to say. It would rather seem that the effect on the nucleus is secondary since the nuclear phenomena accompanying the *first* cleavage appear to be unaffected.

Summary

1. Ultra-violet radiation either by the full arc for 1/10 to 10 seconds or by monochromatic radiation of 2260–2480A for two to eight minutes causes activation in normal unfertilized eggs of *Arbacia punctulata*, in the centrifuged whole eggs and in the halves and quarters derived from the whole eggs by centrifugal force.

2. Cleavages of the eggs and fractions have been obtained up to a 12- to 16-cell stage.

3. The nuclear phenomena of the nucleate eggs seem to be normal for the first cleavage, but in later cleavages many abnormal mitoses occur, chiefly multipolar and anastral.

4. Fractions of eggs without nuclei are activated by ultra-violet radiation just as they are by hypertonic sea water. These parthenogenetic merogones cleave and go to a 12-cell stage quite similar to that of a nucleate egg.

5. The ultra-violet radiation affects, therefore, primarily the cytoplasm.

REFERENCES

Harvey, E. B., 1932. The development of half and quarter eggs of Arbacia punctulata and of strongly centrifuged whole eggs. *Biol. Bull.*, **62**: 155.

Harvey, E. B., 1936. Parthenogenetic merogony or cleavage without nuclei in Arbacia punctulata. *Biol. Bull.*, **71**: 101.

Hollaender, A., 1938. Monochromatic ultraviolet radiation as an activating agent for the eggs of Arbacia punctulata. *Biol. Bull.*, **75**: 248.